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SEGREGATION OF COLONY ODOR IN THE DESERT ANT *Cataglyphis niger*

SIGAL LAHAV,[1] VICTORIA SOROKER,[1,3] ROBERT K. VANDER MEER,[2] and ABRAHAM HEFETZ[1,*]

[1]*Department of Zoology*
George S. Wise Faculty of Life Sciences, Tel-Aviv University
Ramat Aviv 69978, Israel

[2]*Center for Medical Agricultural and Veterinary Entomology*
US Department of Agriculture, Agricultural Research Service
1600 SW 23 Drive, Gainesville, Florida 32608

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Abstract—There are two separate, and presumably opposing, processes that affect colony odor in the desert ant *Cataglyphis niger*: (1) biosynthesis and turnover of these chemicals by individual ants, and (2) homogenization of colony odor through exchange of cues. The first increases signal variability; the latter decreases it. The impact of these factors was tested by splitting colonies and monitoring the profile changes occurring in the postpharyngeal glands (PPG) and cuticular hydrocarbons.

From each of two polygynous nests four daughter colonies were formed, three monogynous and one queenless. Thereafter, 10 ants from each were randomly selected each month, for three successive months, for analyses of their PPG and cuticular hydrocarbons. From two colonies we also obtained ants from a known matriline. Over time, there was a shift in hydrocarbon profiles of both the PPG and cuticular washes in each of the tested colonies. Moreover, by subjecting selected hydrocarbon constituents to a discriminant analyses based on their relative proportions, all of the daughter colonies (queenright and queenless) were distinguishable from each other and from their respective mother colonies. In each of the queenright daughter colonies, the queen profile was indiscriminable from that of the workers and often was in the center of the group. Full sisters were clearly distinguishable from their nestmates, emphasizing the genetic versus environmental processes that govern colony odor. The effect of time was always superior to the separation effect in contributing to odor segregation. Comparison of the Mahalanobis distances indicated that the shift in hydrocarbon seems to proceed along parallel lines rather than in divergence. However, there was no

[*] To whom correspondence should be addressed.
[3] Current address: Department of Entomology, Institute of Plant Protection, Volcani Center, Beit Dagan, Israel.

overt aggression between ants that originated from the different subgroups in dyadic encounters. It appears that in this species a three-month separation period is not sufficient to change the hydrocarbon profile beyond the recognition threshold.

Key Words—Hydrocarbons, *Cataglyphys niger*, nestmate recognition, postpharyngeal gland, gestalt.

INTRODUCTION

Studies pertaining to nestmate recognition have focused in recent years on the nature of the recognition cues and the modes by which colony odor is obtained. In ants, and to some extent in termites, we have indirect, correlative evidence on the role of cuticular hydrocarbons (HCs) as recognition cues (Bonavita-Cougourdan et al., 1987a,b, 1993). Direct evidence for the specific involvement of HCs, but not the more polar cuticular lipids, in interspecific recognition was obtained for *Reticulitermes* (Bagnères et al., 1991), and for intraspecific nestmate recognition in *Cataglyphis niger* (Lahav et al., 1999).

The finding that cuticular HC composition appears congruent with that of the postpharyngeal gland (PPG) (Bagnères and Morgan, 1991), has focused research on the role of this gland in nestmate recognition. PPG extracts were indeed able to modify aggressive behavior in two phylogenetically remote species, *Manica rubida* (Hefetz et al., 1996) and *C. niger* (Soroker et al., 1994). Using HCs as a model for recognition cues and *C. niger* as a model ant, it was further demonstrated that the PPG acts as a "gestalt organ" that enables the admixing and rapid integration of odors from various sources (Soroker et al., 1994, 1995b). This provided empirical evidence supporting the "gestalt model" for creation and acquisition of a general colony odor (Crozier and Dix, 1979). At the individual ant level, there is an exchange between epicuticle and PPG HCs through self-grooming, which explains the chemical congruency between them. This was also confirmed for *Camponotus vagus*, where deposition of (Z)-9-tricosene on the cuticle of a worker resulted in its occurrence in the PPG (Meskali et al., 1995). At the colony level, mutual exchanges of cuticular and PPG HCs between nestmates takes place through trophallaxis, allogrooming, and physical contact. This was determined for several species belonging to remote ant subfamilies, as well as for artificially mixed species groups (Soroker et al., 1995b). Evidence for HC exchange was also observed in a species of termite, but the exchange was achieved only through physical contact (Vauchot et al., 1996, 1998). Blending of individual odors into a uniform colony odor seems to be a system common to social insects.

There are two separate processes that presumably govern the chemical nature of the recognition signal within ant colonies: (1) biosynthesis and turnover of these chemicals by each individual in the colony, and (2) the homogenization of colony odor through continuous exchange of recognition cues. These opposing factors affect the variability of the chemical signal. Individual production increases variability, whereas signal exchange decreases it. Signal production by each individual is probably genetically based, and we can assume that it remains qualitatively constant, although it has been shown in other ant species that quantitative changes (relative amounts) in cues can occur over time (Vander Meer et al., 1989; Provost et al., 1993; Boulay et al., 2000). In polygynous colonies variability in signal composition is increased by the cohabitation of several matrilines (and possibly several patrilines within each matriline). Despite this individual variability in signal composition, a unified colony odor is maintained by mutual exchanges of the signal among members of the colony. Because population composition of a given colony changes with time, we hypothesized that colony odor is dynamic and also changes with time. We tested this hypothesis by following the changes in HC profiles with time in polygynous mother colonies of *C. niger*, as well as their monogynous or queenless daughter colonies.

METHODS AND MATERIALS

Collection and Maintenance of Ant Colonies. Two polygyne colonies of *C. niger*, A (with 11 queens) and B (with 18 queens), were collected from the Tel-Aviv area, transferred to artificial nests in the laboratory and reared under controlled conditions ($28 \pm 2°C$, 8L : 16D photoperiod). All colonies were provided with an identical diet of sugar water and minced insects three times a week. Three monogyne fragments and one queenless (QL) group (daughter colonies) were created from each of the two polygyne colonies (mother colonies) (Figure 1). The mother and daughter colonies were then separated for three months. Each original mother colony consisted of about 3000 ants, and each daughter colony of 250 ants. Before separation a random sample of 10 ants was taken from each of the mother colonies for chemical analyses of the PPG and cuticular washes. Thereafter, once a month, for three months, 10 ants were sampled from the mother colonies and from one of the daughter colonies for chemical analyses. The three other daughter nests were sampled after three months of separation. From two monogyne daughter colonies (A1 and A3) matriline workers were obtained. These were separated from their respective colony as pupae and the emerging adults (manually assisted) were analyzed separately at the age of seven days.

Chemical Analysis. Detailed chemical analyses of PPG and cuticular HC profiles have been published previously (Soroker et al., 1995a; Soroker and Hefetz, 2000). A sample from each mother colony was analyzed by GC-MS to verify that HC profiles from workers of the two mother colonies were qualitatively identical to previously published results.

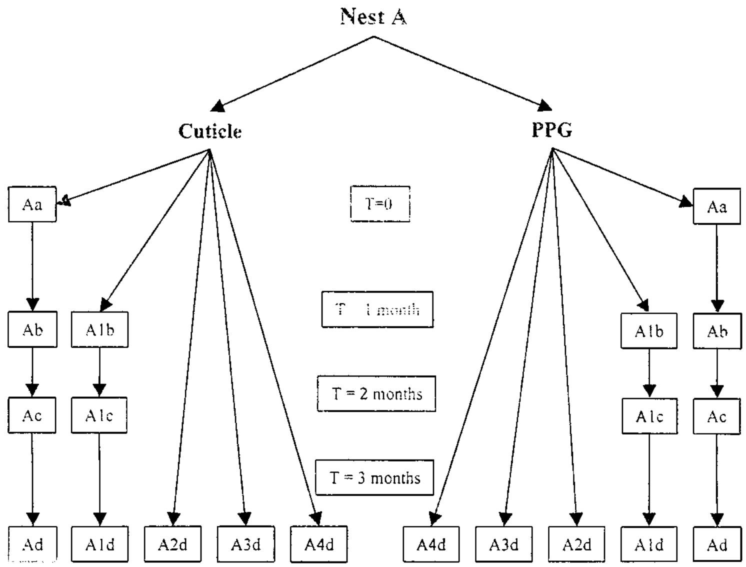

FIG. 1. Flow chart showing the procedures of nest separation and chemical analyses of PPG extract and cuticular washes of ants from a polygyne mother nest and its daughters' monogyne or queenless fragments. Abbreviations are as follows: Aa, mother nest at time 0, before the separation; Ab–Ad, mother nest after one, two, and three months, respectively; A1b–A1d, daughter nest after one, two, and three months, respectively; A2d, A3d, A4d, daughter colonies after three months of separation.

Extraction was accomplished by immersing dissected glands or isolated thoraces in 100 μl of pentane. Thoraces were extracted for 5 min only, to avoid internal contamination. Eicosane (750 ng) was added to each as an internal standard. Samples were run on a Varian 3700 gas chromatograph (Varian Analytical Systems, Walnut Creek, California) equipped with a split/splitless injector, a flame ionization detector, a Leap Technologies autosampler, and a fused silica capillary column (30-m DB-1, 0.32 mm ID, 0.25-μm film thickness capillary column; J&W Scientific Incorporated, Folsom, California). The oven was temperature programmed from 120°C to 285°C at 5°C/min. The carrier gas was hydrogen and the make-up gas nitrogen. Quantification of the various components was achieved by peak integration in comparison to the internal standard using the TurboChrome Workstation, software version 6.1.0.1:F04 (Perkin Elmer Corporation, Norwalk, Connecticut).

Statistical Analysis. To assure equal treatment of the data we selected 18 HC peaks (out of a total of 72) that could be accurately and reliably quantified (Figure 2) and performed a canonical discriminant analysis, using the relative proportions of each of the above-mentioned peaks. This analysis takes a classification variable (mother or daughter colony) and quantitative variables (the selected

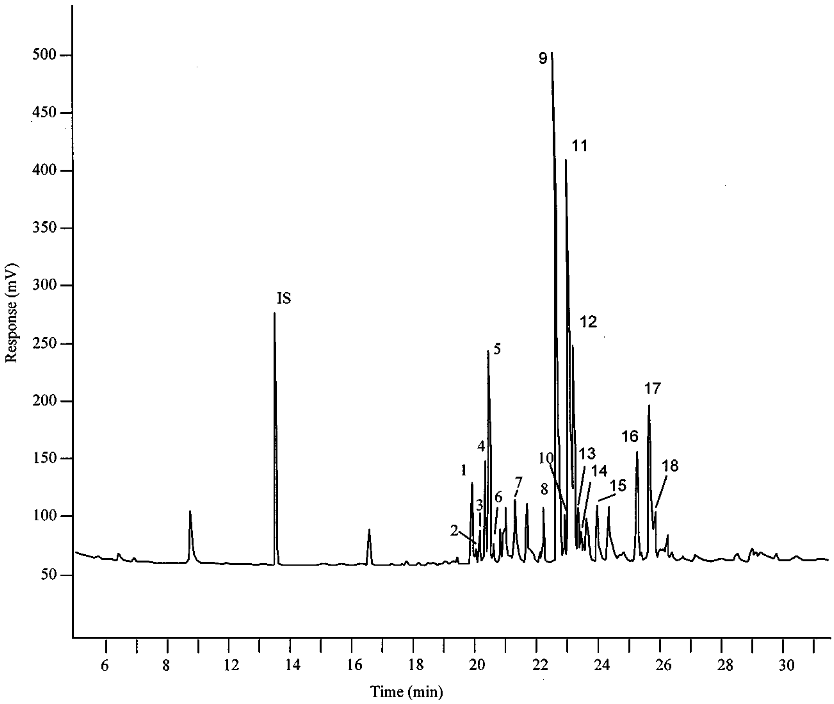

FIG. 2. Gas chromatogram of PPG secretion of *C. niger* workers delineating the peaks used in the discriminant analyses to assess the effects of time and social environment on hydrocarbon profile expression. Peak numbers are designated as follows: 1, 13- + 11-methylheptacosane; 2, 7-methylheptacosane; 3, 5-methylheptacosane; 4, 11,15-dimethylheptacosane; 5, 3-methylheptacosane; 6, 5, *x*-dimethylheptacosane; 7, 12-methyloctacosane; 8, *n*-nonacosane; 9, 9- + 11- + 13- + 15-methylnonacosane; 10, 5-methylnonacosane; 11, 11, 15- + 11, 17- + 11, 19- + 9, 13-dimethylnonacosane; 12, 3-methylnonacosane; 13, 5, 13-dimethylnonacosane + 5, 9-dimethylnonacosane; 14, *x*, *y*, *z*-trimethylnonacosane; 15, *x*, *y*-dimethyltriacontane; 16, 11- + 13- + 15-methylhentriacontane; 17, 11, 15-dimethylhentriacotane; 18, 7, 15-dimethylhentriacontane.

18 peaks from each chromatogram) and derives a canonical variable that summarizes between-class variation. The statistical analysis was performed using Statistica 5.0 for IBM.

Behavior Assays. Behavioral assays were carried out between workers of the subgroups after three months of separation, to test whether the separation had an affect on nestmate recognition. Dyadic encounters between workers from a daughter colony and workers from their respective mother colony were conducted ($N = 19$ for each colony). Encounters between nestmates from the daughter as well as the mother colony served as a control ($N = 15$ for each of the four respective colonies). The behavior of the ants was registered every 3 min for the first 3 hr of the encounter. The protocols for registering the behavior and construction of the aggression index were as previously published (Lahav et al., 1999).

RESULTS

Figures 3–6 present the results of discriminant analyses performed on the HC profiles (based on the selected 18 peaks) for colonies A and B. In general, the within-group compositions of cuticular extracts were more heterogeneous than those of the PPG extracts. Consequently, the groups were less separated using cuticular hydrocarbons when compared to analyses using PPG extracts. This was expressed by the consistently higher values of Wilk's lambda (a measure of the extent of separation among the groups tested and displayed in each of the discriminant analysis graphs). Nonetheless, in all cases the between-group separation was significant ($P < 0.001$, ANOVA) for both the PPG and cuticular extracts. Figure 3 depicts the differences in HC profiles of PPG and cuticle, separately, of the mother colonies A and B and their daughter fragments. In both nests, regarding the HC profiles of PPG, the mother colonies were clearly separated from the daughter colonies. Separation between daughter colonies was less pronounced, with some overlap of the 95% confidence limit ellipsoids. The between-group separations, however, were significant and the centroids were clearly distinct. Two points should be emphasized: (1) the ants from the queenless daughter fragment were clustered and well separated from the queenright daughter fragments, suggesting that their homogeneity was not inferior to that of the queenright daughter fragments; and (2) queens (marked with an arrow and the letter Q in Figures 3, 5, and 6) of the queenright daughter colonies were often the closest to the group centroid (except for the queens of daughter colonies 1 and 2 for colonies A and B, respectively). Although the homogeneity of the cuticular profiles was less apparent, albeit the between-group separation was significant, the same trends as seen for the PPG occurred.

Figures 4 and 5 depict temporal changes in the HC pattern of both mother and daughter colonies for which ants were sampled and analyzed monthly. In these figures $T = 0$ is representative of the HC profile (PPG and cuticle, respectively) of the mother colonies A and B before the queenright and queenless fragments were created. Figure 4 shows a clear shift in PPG HC profiles of ants sampled from the mother colonies at each of the time points analyzed. Although the direction of changes can not be determined, it can be seen that the changes in colony A were of similar magnitude every month. In colony B the changes were not as symmetrical, and those between months 2 and 3 were slightly less pronounced. Cuticular profiles also changed with time in the mother colonies, but again this was less pronounced. Similar changes with time were observed in the two queenright daughter colonies (Figure 5) for which monthly samples of ants were analyzed.

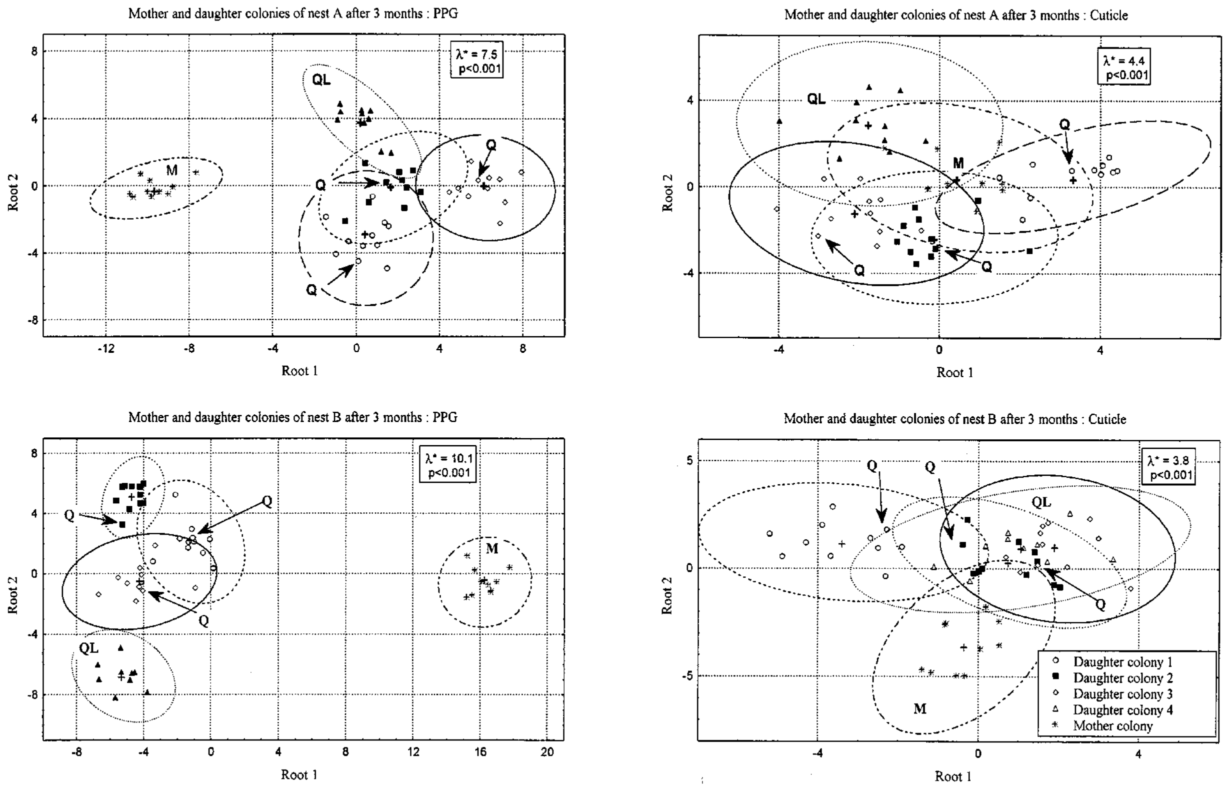

FIG. 3. Signal segregation estimated by a discriminant analysis based on hydrocarbon profiles of the polygyne mother nests and the monogyne queenright or queenless daughter colonies after three months of separation. The ellipses around each group delineate the 95% confidence limits. λ^* was calculated from Wilk's λ after Ln $1/\lambda$ transformation for linearity. The centroid of each group is marked by a plus sign. The HC profile of the queen in each of the queenright daughter groups is marked by an arrow and the letter Q. The queens were considered as a member of the appropriate daughter group for the discriminant analyses.

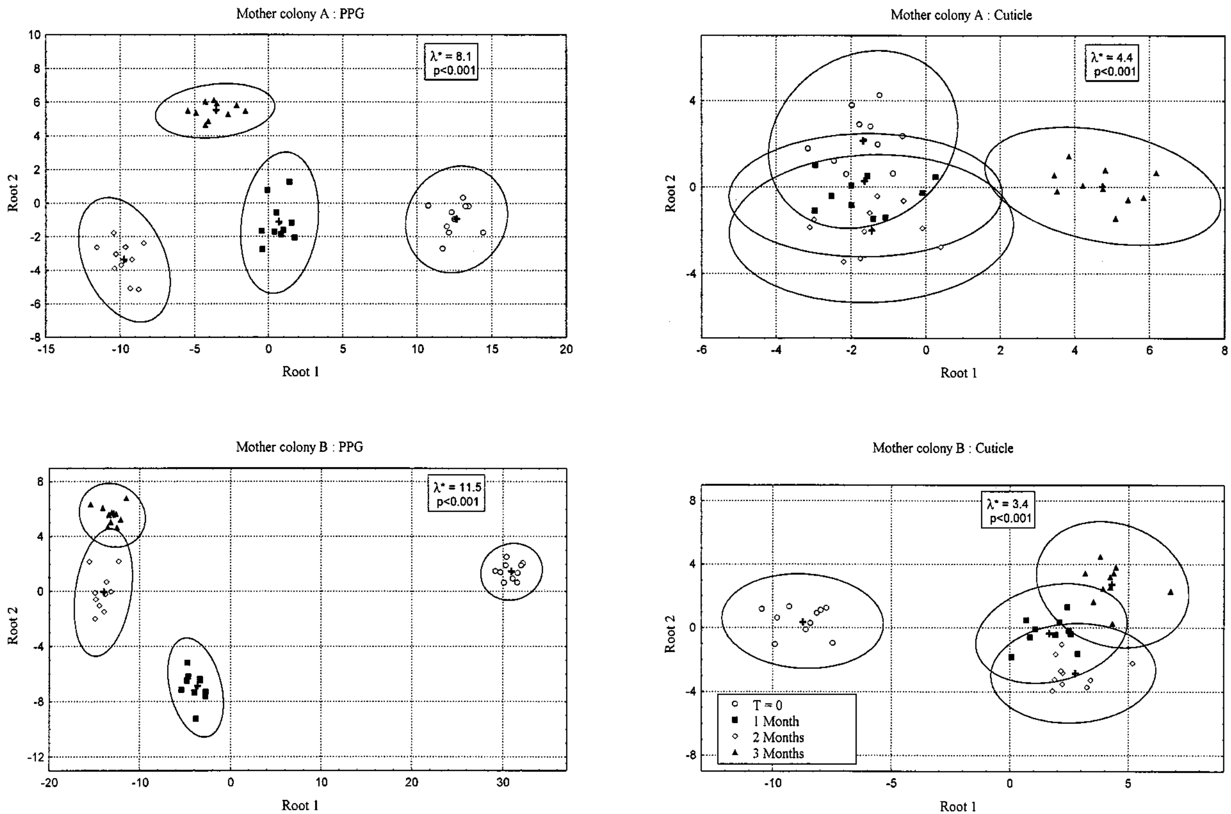

FIG. 4. Time-dependent signal segregation estimated by a discriminant analysis based on hydrocarbon profiles of the polygyne mother nests A and B. $T = 0$ represents the mother colony before it was split. Thereafter the mother nests were sampled after one, two, and three months. The centroid of each group is marked by a +.

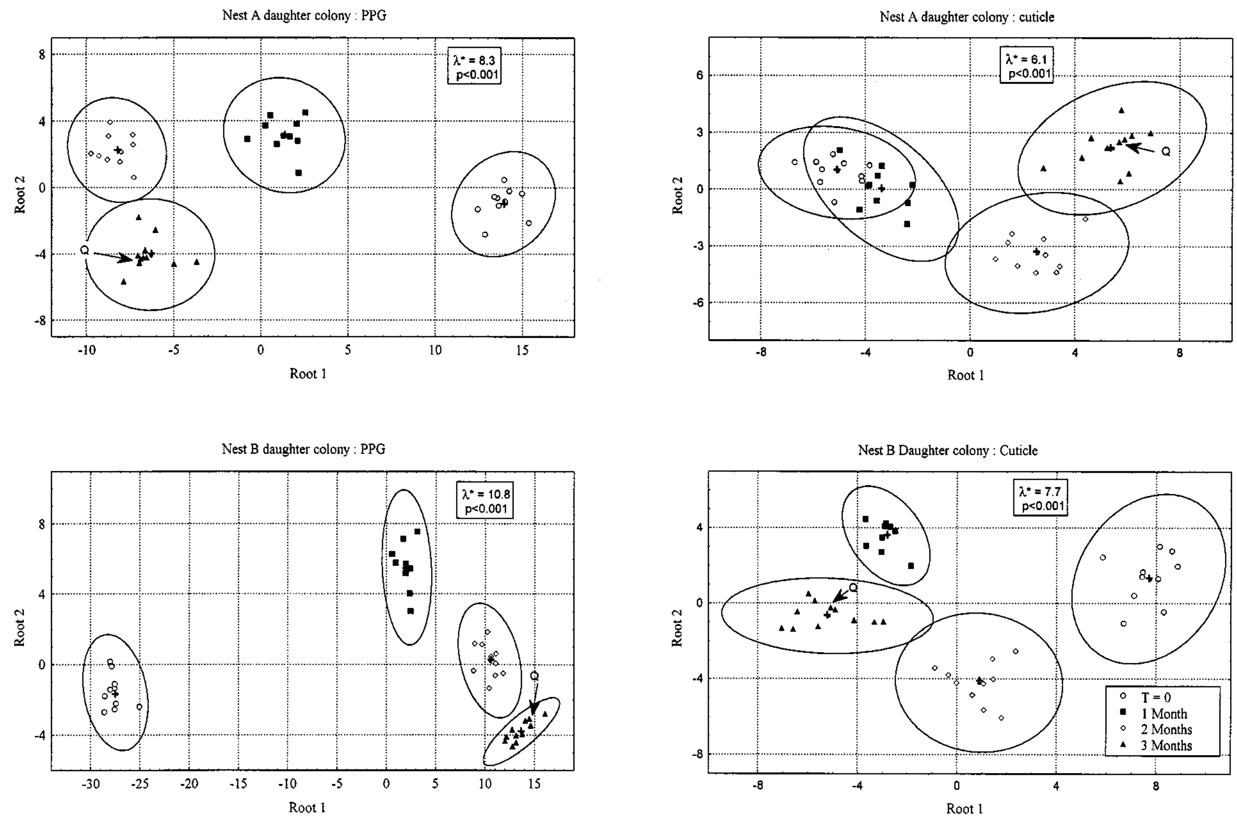

FIG. 5. Time-dependent signal segregation estimated by a discriminant analysis based on hydrocarbon profiles of the monogyne daughter nests. $T = 0$ represents a sample of the mother colonies at the onset of the experiment before separation. Thereafter, the daughter colonies were sampled after one, two, and three months. The centroid of each group is marked by a plus sign. The HCs profile of the queen in each of the queenright daughter groups is marked by an arrow and the letter Q. The queens were considered as a member of the appropriate daughter group for the discriminant analyses.

In this case separation of both PPG profiles and cuticular profiles was good. For these colonies the queen was killed at the end of three months. Both queen HC profiles (PPG and cuticle) were near the centroid (Figure 5).

Figure 6 shows HC profile similarities between a group of matrilines and their randomly selected nestmates. In this figure we include the profile of ants from the mother colonies at time 0 as a reference point. As can be seen, the matriline HC profiles are grouped together and are distinctly separated from their nestmate. For both cuticle and PPG HC profiles the corresponding colony queen profile of each colony was positioned in between the profiles of the matriline and colony worker groups.

The above discriminant analyses represent a combined effect of the changes with time and the separation from the mother colony. In order to investigate the relative impact of each of these factors we compared the mean squared Mahalanobis distance (MSMD) between the HC profile of one group and the centroid of a second group (Table 1). Greater MSMD corresponds to larger deviations of the HC profiles between the groups. For example, the MSMD between group Aa (mother colony at $T = 0$) and the centroid of group A1b (daughter colony 1 after 1 month) represent the divergence of the daughter colony profile after one month from that of the mother colony at $T = 0$. This represents the combined effect of colony separation and the time factor. On the other hand, comparing Aa with Ab (mother colony after one month) represents the effect of time only on profile divergence. Likewise, comparing the profiles of Ab and A1b (mother and daughter colonies respectively after one month) represents the effect of group separation on profile divergence. Table 1 presents the results of such analyses over the three-month period of the experiment for colonies A and B. For both colonies the MSMD was consistently highest when both time and separation were taken into account. Of the two factors, time was the most influential in all cases, as revealed by its larger MSMD, when compared to the separation factor. The fact that the physical separation between the colonies caused the lowest shift in HC profiles suggests that the profiles of each group changed in a similar way, i.e., it reflects the genetic similarity between the groups. To test this hypothesis, the MSMD was plotted as a function of the time of separation for these factors (time and separation) alone or combined. As depicted in Figure 7, the lowest slope is for the separation factor, and for colony B there was no slope at all. Although the sample size was small, these results suggest that, although each of the colony HC profiles changed with time, the magnitude of the changes was similar rather than divergent.

Behavioral Assays. In all the encounters performed, aggressive behavior was rare and limited to mandibular opening. The level of aggression of workers from the mother colony towards workers from the daughter colony was 0.08 ± 0.03. The aggression of workers from the daughter colonies towards workers from the mother colonies was similarly low, amounting to 0.11 ± 0.04. Neither was statistically

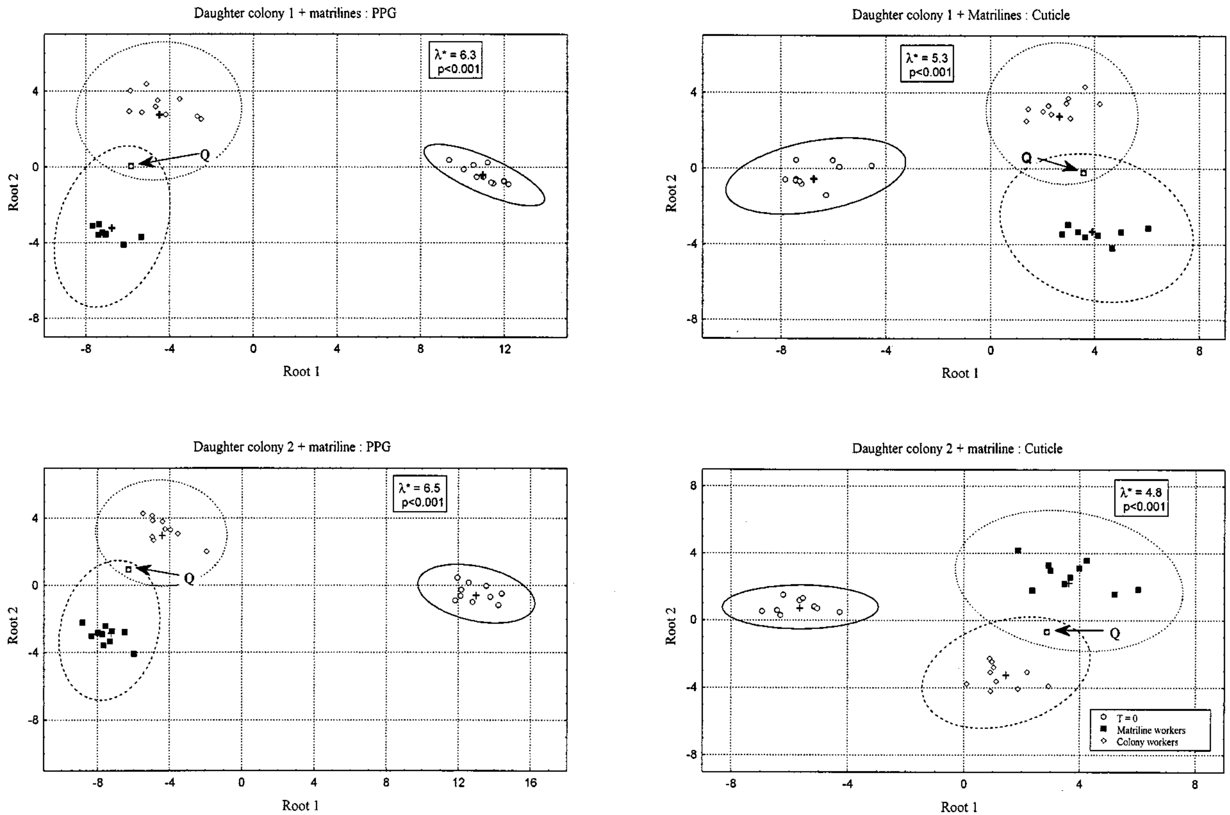

FIG. 6. Signal segregation estimated by a discriminant analysis based on hydrocarbon profile of the polygyne mother nest A at $T = 0$ and two of its monogyne daughter colonies (1 and 2, respectively). The daughter colonies constituted two groups—random workers that originated from the polygyne mother colony and matriline workers that were laid by the respective queens and that emerged in isolation.

TABLE 1. EFFECT OF GROUP SEPARATION AND TEMPORAL CHANGES ON HC PROFILE DIVERGENCE IN *C. niger* WORKERS[a]

Group	Factors affecting profile divergence	Time of separation (mo)	Mean squared Mahalanobis distance	
			PPG	Cuticle
Nest A				
Aa-Alb	Time + separation		139.7 ± 27.1	27.1 ± 10.1
Aa-Ab	Time	1	88.1 ± 17.4	24.1 ± 7.6
Ab-Alb	Separation		44.4 ± 12.4	33.9 ± 25.8
Aa-Alc	Time + separation		280.5 ± 23.5	33.6 ± 8.5
Aa-Ac	Time	2	222.2 ± 24.6	24.7 ± 10.0
Ac-Alc	Separation		124.7 ± 18.3	22.4 ± 10.8
Aa-Ald	Time + separation		236.5 ± 27.6	64.1 ± 11.6
Aa-Ad	Time	3	177.4 ± 22.3	41.8 ± 9.4
Ad-Ald	Separation		103.7 ± 6.3	30.8 ± 18.7
Nest B				
Ba-Blb	Time + separation		767.7 ± 52.6	64.1 ± 11.6
Ba-Bb	Time	1	474.5 ± 40.6	68.9 ± 23.7
Bb-Blb	Separation		83.3 ± 28.7	46.1 ± 16.6
Ba-Blc	Time + separation		1108.0 ± 56.9	67.3 ± 24.4
Ba-Bc	Time	2	852.9 ± 51.6	76.4 ± 23.5
Bc-Blc	Separation		54.7 ± 13.6	25.3 ± 9.1
Ba-Bld	Time + separation		1133.8 ± 58.6	101.4 ± 28.1
Ba-Bd	Time	3	869.9 ± 49.8	104.9 ± 30.0
Bd-Bld	Separation		71.2 ± 21.6	37.9 ± 23.7

[a] Values are expressed a mean squared Mahalanobis distance (MSMD), obtained from the discriminant analyses, between individuals of selected groups and the centroid of the appropriate second group.

different from aggression between nestmates, which equaled 0.13 ± 0.04 (pooled data from the mother and daughter colonies; Mann-Whitney $P = 0.38$).

DISCUSSION

The temporal chemical analyses revealed that HC profiles of colonies of *C. niger* appear to shift with time. This was true irrespective of number of queens in the colony, polygyne, monogyne, or queenless. The results corroborate earlier findings in several ant species, e.g., *Solenopsis invicta* (Vander Meer et al., 1989), *Leptothorax lichtensteini* (Provost et al., 1993), *Cataglyphis iberica* (Dahbi and Lenoir, 1998), and *Formica truncorum* (Liebig et al., 2000). Since the rearing conditions were constant during the three months of separation and only a few new workers emerged, we can exclude the possibility that changes in HC profiles over time are due to the physical environment and/or population changes. The ants were sampled at random and were all well matured workers, also excluding a possible

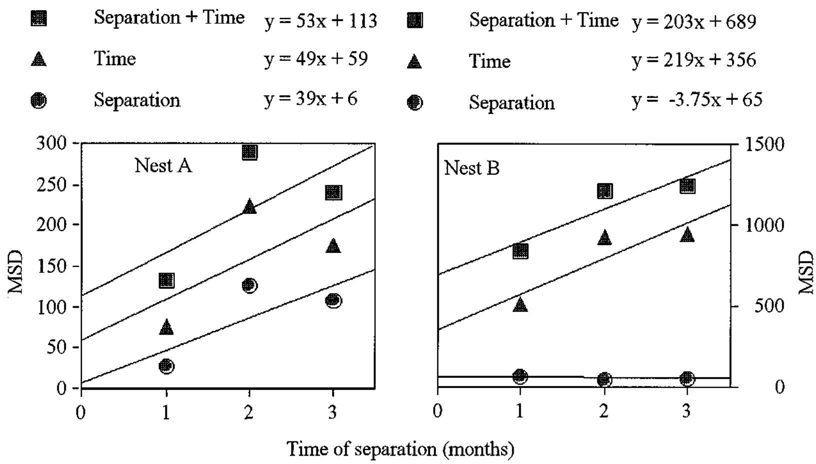

FIG. 7. Changes in Mahalanobis distance as a function of time of separation. The slopes delineate the effects of the factors time and separation combined, time alone, and separation alone.

age effect on the HC composition. We suggest, rather, that these changes are the result of genetically based changes in HC expression. These are not qualitative differences, i.e., disappearance or appearance of specific compounds, but quantitative changes expressed as shifts in the relative intensities of existing compounds. Because in polygyne *C. niger* genetic heterogeneity is large, it was predicted that individual differences in HC composition would create divergent odor compositions in the individuals within a group; however, within-group homogeneity was clear for all groups.

The PPG has been postulated to be the nestmate recognition "gestalt" organ (Soroker et al., 1994, 1995b), where heritable and environmental contributors to colony odor (including recognition cues) are continuously blended and dispersed through trophallaxis. Recent time-course experiments revealed that at any one time the PPG content is more mobile and changes faster than that of the cuticle and that the rate of PPG content change is dependent on the rate of trophallaxis exhibited by the species (Lenoir et al., 2001). Our data clearly support a "gestalt" organ function for the PPG. Similar results were obtained in *C. iberica*, where groups of workers separated from their mother nest for five months exhibited differences in their PPG HC profiles, while remaining homogenous within each group (Dahbi and Lenoir, 1998). It was also demonstrated in this species that when isolated groups of nestmates are reunited, trophallaxis between groups was significantly higher than trophallaxis within group (Dahbi et al., 1999), which would be expected to lead to homogenization of the recognition cues associated with each of the reunited groups.

A recent study corroborates the assumption that changes in HC compositions are individual and may lead to divergent odor composition. Worker *Camponotus fellah* ants that were isolated for up to 40 days showed a higher mean Euclidean distance between individual HC profiles than their nestmates that were reared in groups (Boulay et al., 2000). In our study the matriline ants were reared separately and each worker could not exchange HCs with any of the others. Nonetheless, they showed within-group homogeneity as good as groups that had the opportunity for social interactions (grooming and trophallaxis). The newly eclosed ants were reared under consistent conditions excluding all possible environmental influence. This suggests that, within the timeframe of our experiment (seven days after eclosion), heritable cuticular HC patterns change in parallel rather than at random. It is unknown whether *C. niger* mates with only one male or with several; however, our results suggest that matrilines in *C. niger* have high genetic relatedness.

The HC composition of the PPG was more homogenous within groups, and between-group separation was better than the corresponding cuticular HC samples. Quantitatively, the cuticle contains larger amounts of *n*-alkanes, in particular *n*-nonacosane, than are present in the PPG (Soroker and Hefetz, 2000). Thus, there is no direct quantitative correspondence between HCs derived from the cuticle and those derived from the PPG. To date, behavioral bioassays have shown a causative nestmate recognition role for total PPG HCs (Lahav et al., 1999), but the role of the specific types of HCs in nestmate recognition is unknown. Therefore, we can not assess whether or not the differences in PPG and cuticular homogeneity have any consequences on nestmate recognition. This indicates that the controlling mechanisms are different for the two systems. We suggest that interaction occurs between the PPG and cuticular lipids (monitored through HC analysis), analogous to an environment/gene interaction.

How the cuticular HC profiles are influenced by the PPG contents is complex and not well understood. There are external and internal mechanisms for the PPG to interact with cuticle chemical profiles. Transfer of material between body surfaces of nestmates is done mainly by allogrooming and/or by self-grooming that takes place after trophallactic exchanges (Soroker et al., 1995b). Internally, the PPG (the "gestalt" organ) has been shown to obtain hydrocarbons from the hemolymph (Soroker et al., 1995b), presumably via lipophorin transport; however, there may be active bidirectional exchange, thus permitting the hydrocarbon content of the PPG to influence the hydrocarbon pattern on the cuticle. The contents of the PPG as colony blend subject to constant updating can be considered as a continuous environmental impactor on the individual worker's heritable cuticular hydrocarbon profile. Our results demonstrate that in this environment (PPG)–genetic (an individual's heritable cuticular hydrocarbon profile) interaction is not perfect, since cuticular hydrocarbon variability is greater than PPG hydrocarbon variability; however, the observed variability is not great enough to cause a behavioral reaction (aggression). The "gestalt" PPG hydrocarbon pool influences, but does not dictate the hydrocarbon composition on the cuticle. Qualitative and quantitative selectivity may occur with lipophorin transport and/or at the site of hydrocarbon uptake at the epicuticle, but the main point is that the cuticle hydrocarbon profile does not have to mimic the PPG composition. The position of the queen's profile in relation to that of her matriline workers and that of her randomly selected nestmates is especially interesting. In both cases the queen's profile was positioned in-between the two worker groups and was almost completely congruent with one of the matriline ants. This illustrates well the factors affecting colony odor composition in the polygyne *C. niger*. Genetic influences create within-matriline uniformity but multiple matrilines yield within-colony heterogeneity, while the continual nondiscriminatory cue exchanges between all workers eventually results in a uniform colony odor.

The contribution of the queen to the changes in HC profile can be deduced indirectly by comparing the monogyne and queenless daughter colonies. The results revealed homogeneity within each daughter colony, whether queenright or queenless. This finding differs from previously reported observations on the queen effect on HC uniformity in *Formica sp.* workers (Yamaoka and Kubo, 1990), and highlights interspecific variability and the dangers of generalization. In *C. niger* we have demonstrated by biochemical means that the direct impact of queen on the composition of colony odor and on nestmate recognition is minor. In fact, the queen donates smaller amounts of HCs than she gets in trophallactic exchanges, which, combined with the very low HC biosynthesis rate exhibited by queen, results in her always being in the center of the gestalt (Lahav et al., 1999). This hypothesis is supported by the present study's findings that queen profile was often in the center of the group.

Divergence in HC profiles did not affect nestmate recognition. Workers separated for three months still reacted to each other as nestmates despite measurable differences in HC patterns. This suggests that the observed differences are still within the cue variation that normally occurs in *C. niger* colonies. Interestingly, in encounters between workers from the separated subcolonies, there were some cases of mutual transport. This behavior was never observed between nonseparated nestmates, indicating that the separated workers may have sensed chemical differences that were not drastic enough to elicit aggression, but were sufficient to induce carrying behavior. A similar phenomenon was found in *C. iberica*, where, after hibernation, transport of callow workers could be correlated with chemical disparity (Dahbi et al., 1997).

Recognition mechanisms involve an interplay between the label (recognition cues) and the mechanism for its detection and interpretation, i.e., the template. It follows that the temporal changes in recognition cues (HCs in the case of *C. niger*) must also affect the characteristics of the template. While the nature of this template is still obscure, we can spectulate on the consequences of the above-shown change in HC profiles. Recognition based on matching self-odor or nestmate odor with the odor of the encountered ant is presumably not affected by our observed temporal shifts in odor. The effective formation of a uniform colony odor on the resident as well as its nestmates buffers these changes, resulting in congruence between the individual odor and that of nestmates at all times. If the template is neural, the temporal changes in referent (cues), regardless of the referent source, self or nestmate, should also be reflected in neural plasticity with regards to the template. Although we only monitored the changes in HC profiles at monthly intervals, we can assume that these changes are continuous rather than abrupt. This, coupled with the continuous homogenization within the colony, should facilitate the constant updating of the experience-based neural template despite the odor shifts within the colony, as demonstrated in this work.

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